RESEARCH ON THE CHANGES OF SOME PHYSIOLOGICAL PARAMETERS IN PRUSSIAN CARP UNDER THE ACTION OF THE FUNGICIDE ANTRACOL

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Abstract. Water is indispensable for all physiological processes that determine the natural, correct development of living organisms. The intense anthropogenic activity diminished water retention capacity and caused increasing pollution to an extent that often threatens public health (NIKONOROV, 1981). The production and use of pesticides is a serious threat to the hydrosphere, which receives large amounts of such chemical substances, because of the waste discharges from the factories of antiparasitic products or because of rainwaters that wash away these substances from the treated agricultural lands (MOHAN & ARDELEAN, 1993). The spread of pesticides has become global as they are present in all inland waters which, in the end, reach different seas and oceans (BREZEANU & SIMON-GRUIȚĂ, 2002). In case of water contaminated with pesticides, the environmental impact is mainly due to the bioconcentration and biomagnification capacity of pesticides in organisms (GAVRILESCU, 2008). The paper renders the results of the investigations referring to the modifications of certain physiological indices under the influence of the fungicide Antracol 70 WP (Propineb 70%) at two thermal thresholds. Temperature is one of the most important environmental factors the living organisms had to adapt to in order to maintain their vital functions at an optimum level (MIHAI & BREZEANU, 2013). Antracol is a contact organic fungicide from the dithiocarbamate group. It is toxic to fish and aquatic invertebrates (KIDD & JAMES, 1991). It produces thyroid cancer in laboratory animals (HASEGAWA et al., 1993). The investigations proved that the fungicide Antracol 70 WP has an inhibitory effect on the oxygen consumption and respiratory rate on the investigated fish species (*Carassius gibelio* Bloch); at the same time, it increased the number of erythrocytes and reduced the glycaemic level having a hypoglycaemic effect. The carried out experiments emphasized that the fungicide Antracol 70 WP is toxic to fish even at very low concentrations.

Keywords: antracol, fish, energetic metabolism, erythrocytes, glycaemia.

Rezumat. Cercetări asupra modificărilor unor parametri fiziologici la caras sub acțiunea fungicidului Antracol. Apa este indispensabilă pentru toate procesele fiziologice care condiționează dezvoltarea naturală, corectă a organismelor vii. Activitatea intensă a omului a diminuat capacitatea de retenție a apelor și a pricinuit creşterea poluării într-o măsură care de multe ori amenință sănătatea populației (NIKONOROW, 1981). Producerea şi folosirea pesticidelor constituie o gravă ameninţare pentru hidrosferă, în care ajung datorită deversărilor de reziduuri de la fabricile de produse antiparazitare sau datorită spălării acestor substanţe de apele de ploaie de pe terenurile agricole tratate (MOHAN & ARDELEAN, 1993). Răspândirea pesticidelor a devenit globală, fiind prezente în toate apele continentale și duse de apele râurilor și fluviilor în mări și oceane (BREZEANU & SIMON-GRUIȚĂ, 2002). În cazul apelor contaminate cu pesticide, impactul ecologic se datorează în principal capacității de bioconcentrare și biomagnificare a pesticidelor din apă în organisme (GAVRILESCU, 2008). Lucrarea prezintă rezultatele investigațiilor referitoare la modificările unor indici fiziologici la pești sub acțiunea fungicidului Antracol 70 WP (propineb 70%) la două niveluri termice. Temperatura constituie unul dintre cei mai importanți factori de mediu la care organismele vii au trebuit să se adapteze pentru a-și asigura menținerea la un nivel optim a funcțiilor vitale (MIHAI & BREZEANU, 2013). Antracolul este un fungicid organic de contact din grupa ditiocarbamaților. Este toxic pentru pești și organismele acvatice nevertebrate (KIDD & JAMES, 1991). Produce cancer tiroidian la animalele de laborator (HASEGAWA et al., 1993). În urma cercetărilor efectuate s-a constatat că fungicidul Antracol 70 WP are efect inhibitor asupra consumului de oxigen şi ritmului respirator la specia de peşti investigată (*Carassius gibelio* Bloch); de asemenea a determinat creşterea numărului de eritrocite şi scăderea nivelului glicemic, având efect hipoglicemiant. Experienţele efectuate ilustrează faptul că fungicidul Antracol 70 WP este toxic pentru pești și în concentrații foarte mici.

Cuvinte cheie: antracol, peşte, metabolism energetic, eritrocite, glicemie.

INTRODUCTION

Water pollution with pesticides is mainly determined by the activity of the industry that manufactures crop protection chemicals, as well as by the massive application of these substances in agriculture and other sectors of the economy.

Numerous research regarding the biological effects of environmental pollution are oriented to detect functional changes in the animal body induced by the action of chemical agents resulting from the industrial technological processes or from farming, which reach the inland waters (DRĂGHICI, 1979).

Contamination of fresh waters by a wide range of pollutants has become a matter of concern in recent decades (VINODHINI & NARAYANAN, 2008). Increased human activities, especially the rapid development of agriculture and industry, have led to a considerable increase in pollution levels that can cause serious and long-lasting effects for all living organisms (SASTRY & SUKLA, 1993; MURUGAN et al., 2008).

Once they reach the aquatic environment, pesticides affect a wide range of non-target organisms, such as invertebrates and fish (OTLUDIL et al., 2004). Aquatic organisms take food together with large amounts of water, depending on their body mass. Certain swallowed pesticides undergo metabolic changes, while others, taking into account their good solubility in the fatty compounds and reduced capacity of biochemical changes, are accumulated. The amount of pesticides accumulated in aquatic organisms is several times higher than their concentrations in water

(NIKONOROW, 1981). As a result of the accumulation of pesticides in the tissues and organs of fish and other hydrobionts, they intoxicate and, in their turn, become sources of pollution.

Irrespective of form, mode of application and activity field, pesticides enter the body especially through the digestive tract of the animals (polluting food), skin and respiratory system (antiparasitic substances).

Fish are the most used in toxicological experiments not only because they represent a valuable component of water production, but also because they are highly sensitive to most toxic substances (MĂLĂCEA, 1969). Gills proved to be very sensitive to toxic substances and, in many cases, there appear different lesions. Histological lesions of gills were reported in rainbow trout exposed to sublethal concentrations of propineb by CAPKIN et al. (2010): lamellar fusion, hyperplasia, vacuolation, epithelial necrosis, cellular hypertrophy, peeling of the gill epithelium.

Antracol 70 WP (propineb 70%) is an organic contact fungicide from the dithiocarbamate group, which blocks the spore germination and penetration of the mycelium of pathogenic fungi in plants. It adheres very well to the surface of the treated organs and has a good residual effect, which occurs over a period of 7-10 days on the treated surface according to local conditions. It is a multisite fungicide that prevents the occurrence of resistant forms. The product shows good resistance to rain. Antracol 70 WP also contains micronutrients necessary for plant growth and development. It belongs to Group IV of toxicity.

MATERIAL AND METHOD

The research was conducted on the Prussian carp (*Carassius gibelio* Bloch), which we sampled from the Olt River (Fig. 1). When choosing the biological material we have considered the physiological state, body integrity and size of the specimens, the sensitivity to toxic substances, as well as the fact that the species easily adapts to the conditions of retention in the aquarium, tolerates high temperatures and shows good resistance to oxygen deficiency having a low lethal hypoxic limit.

The introduction of fish in the aquariums was done about a month before the start of the experiments, to live them enough time to adapt to the new conditions. The aquariums used during the experiments had a capacity of poisoning of 20-30 litres and were equipped with lighting, stirring and aeration systems. These tanks were cleaned and sanitized. In case of the variants made at 5-7ºC, fish were placed in refrigerators, lighting being artificial. The solutions of toxic substances from the aquariums were renewed at intervals of 24 hours.

The specimens of Prussian carp used in different experimental variants were selected and sorted according to the weight category in order to avoid or, on the contrary, enhance, the effect of the individual factor represented by body weight. We used fish lots consisting in ten specimens each.

Of the three methods for determining the amount of dissolved oxygen (iodometric, gasometric and polarographic method), we used Winkler iodometric method in the achieved experiments, as described by the author at the end of the last century (1888). This method is considered the safest way to quantitatively assess the content of dissolved oxygen in water, under various temperature conditions. To assess the oxygen consumption it was used the technique of confined space described by Stroganov (1962), which allows to easily determine the exact quantity of the oxygen consumed by fish within a known time, based on the difference between the amount of oxygen measured in a control tank and the amount determined at the end of the period (quoted by PICOŞ & NĂSTĂSESCU, 1988).

The measurement of the respiratory rate was performed by successive determinations by means of a timer (their arithmetic mean representing the respiratory rate at the time).

The experiments that required series performed in different days were always made within a nyctemeral as narrow as possible (same time each day), in order to avoid any possible influence of the circadian variations of the oxygen consumption and respiratory rate.

As we aimed at achieving experimental conditions as close as possible to natural conditions, there were kept and used in the experiment only the fish specimens found in the conditions of natural photoperiodism. Although it was not demonstrated a direct relationship between energy metabolism and light, it was taken into account the importance of the light factor in triggering certain functional manifestations.

Much attention was paid to the effect of fish handling during the pre-experimental stage, which may alter the results of the determinations (FRY, 1957; MARINESCU, 1972). For the experiments carried out by the technique based on the principle of confined space, where it is not possible to entirely avoid perturbing fish due to manipulation, we tried to limit its action. Consequently, for the experiment, we chose fish species characterized by low mobility (gibel carp) and we always performed control samples before the actual experiment.

For the experiments carried out to determine the change in the number of erythrocytes and blood glucose levels under the action of the fungicide Antracol 70 WP, there were taken blood samples from the fish specimens. Blood samples were taken from the caudal artery. The number of erythrocytes was determined using Thoma counting chamber (PICOŞ & NĂSTĂSESCU, 1988).

The determination of the blood glucose level was performed using Accutrend GCT device that allows the measurement of its value in a drop of blood on a testing strip in a very short time. All the determinations performed on fish were carried out under strict control. There were eight experimental variants designed to determine the four physiological indices.

Measurements were made after 14 days of treatment at two thermal levels: 5-7ºC and 20-22ºC. The concentrations of Antracol 70 WP used in this work are 2, 4, 8 and 16 mg/l respectively 1.4, 2.8, 5.6 and 11.2 mg propineb/l.

Figure 1. *Carassius gibelio* (Bloch, 1783) (original).

RESULTS AND DISCUSSIONS

1. The effect of the fungicide Antracol 70 WP upon the oxygen consumption

The variation of the oxygen consumption at the Prussian carps exposed to various concentrations of Antracol 70 WP at a temperature of 20-22°C is rendered in Fig. 2.

The evolution of the oxygen consumption at the Prussian carps treated with the fungicide Antracol 70 WP is decreasing. The decreasing trend is linear, the oxygen consumption decreasing with the increase of the application time of the fungicide (R2 = 0.91).

By the application of the test Anova: Two Factor without Replication, there are emphasized statistically significant differences between treatments as concentrations, as well as between the duration of the treatments with the fungicide Antracol 70 WP (p <0.0001, F> Fcrit).

At the end of the experiment (14 days), the percentage decrease of the oxygen consumption was approximately 28-38% compared with that determined prior to the introduction of the fish into the toxic substance (control sample) in increasing order according to concentration.

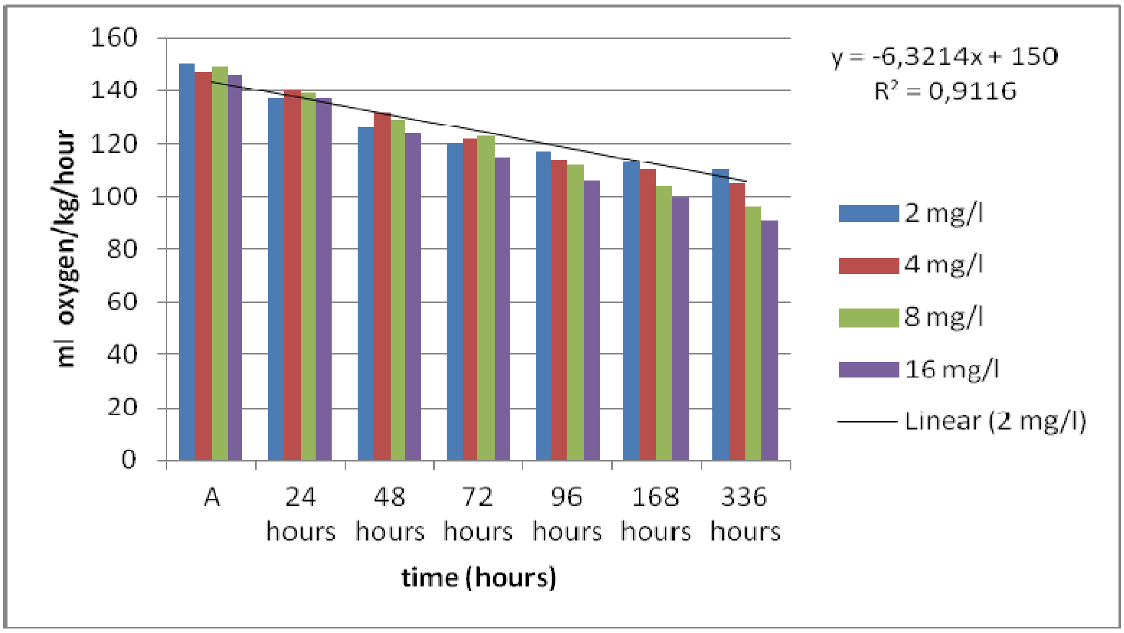

Figure 2. Variation of the average oxygen consumption at the Prussian carp specimens exposed to the action of the fungicide Antracol 70 WP at different concentrations at 20-22°C.

At low temperatures, the toxic effect of the fungicide is slightly weaker (Fig. 3). In this case as well, the trend is decreasing, linear, the oxygen consumption decreasing as the application time of Antracol 70 WP fungicide increases (R2 = 0.98). The reduction is of 24-26% for the first used concentrations (2 and 4 mg / l) compared to the control sample.

For the other two concentrations (8 and 16 mg / l), the effect of the fungicide on the oxygen consumption of the Prussian carp is more intense than the first two ones, the values determined at the end of the experiments being 30-34% lower than the consumption registered before the introduction of fish in the toxic substance.

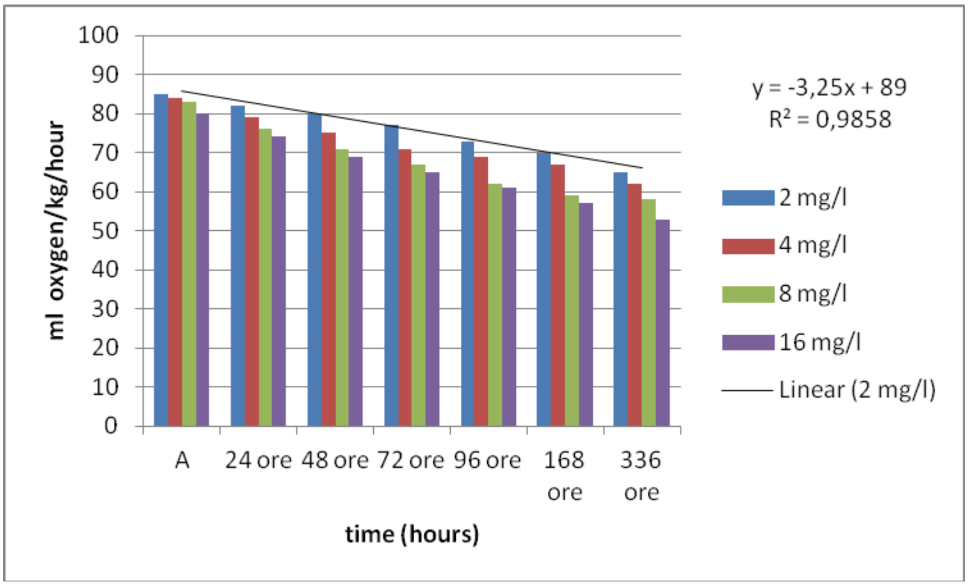

Figure 3. Variation of the average oxygen consumption at the Prussian carp specimens exposed to the action of the fungicide Antracol 70 WP at different concentrations at 5-7°C.

All treatments show statistically significant differences according to time. ANOVA single factor test was applied for each temperature range partially obtaining $p < 0.05$ and F>F crit.

By applying ANOVA Two-Factor without Replication test it results that both time and temperatures influenced statistically significantly the oxygen consumption when applying the fungicide Antracol 70 WP, the reduction of the oxygen consumption being more pronounced at 20-22°C.

2. The effect of the fungicide Antracol 70 WP upon the respiratory rate

The effect of the fungicide Antracol 70 WP on the frequency of the respiratory rate in the Prussian carp for the variants achieved at a temperature of 20-22°C is rendered in Fig. 4. There are statistically significant trends of reduction of the number of breaths per minute with the increase of the application time of the fungicide Antracol 70 WP (R2 = 0.77).

By applying ANOVA Two-Factor without Replication test it can be concluded that there are differences both in terms of number of hours and fungicide concentration ($p < 0.0001$).

In case of the first concentration, the effect is slight inhibitory and it increases in intensity at higher concentrations. The percentage reduction reaches 27% compared to the control sample at the concentration of 16 mg / l.

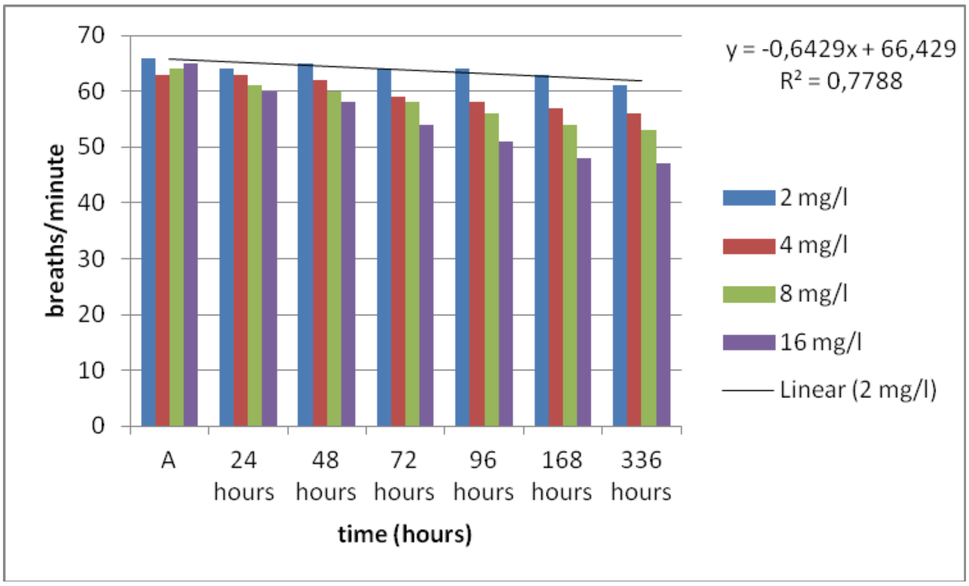

Figure 4. Variation of the respiratory rate at the Prussian carp specimens exposed to the action of the fungicide Antracol 70 WP at different concentrations at 20-22°C.

At a temperature of 5-7°C, the inhibitory effect of the fungicide is weaker compared to that registered at the temperature of the 20-22°C for all concentrations, but more intense for the concentrations of 8 and 16 mg / l (Fig. 5).

There are also registered statistically significant trends of decrease of the number of breaths per minute with the increase of the application time of the fungicide Antracol 70 WP (R2 = 0.94).

By applying ANOVA Two-Factor without Replication test, it can be concluded that there are differences both in terms of number of hours and fungicide concentration (p <0.0001).

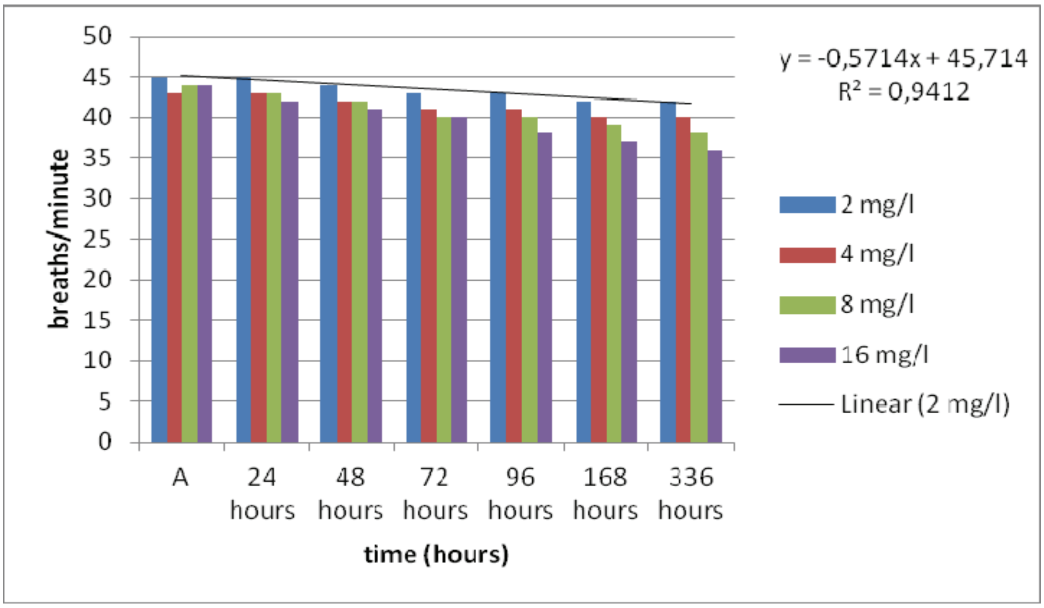

Figure 5. Variation of the respiratory rate at the Prussian carp specimens exposed to the action of the fungicide Antracol 70 WP at different concentrations at 5-7°C.

3. The effect of the fungicide Antracol 70 WP upon the number of erythrocytes

The fungicide Antracol 70 WP has a stimulating effect upon the number of erythrocytes for both thermal thresholds (Figs. 6; 7). The application of the fungicide Antracol 70 WP leads to statistically significant results compared to the control groups in terms of the number of erythrocytes. This is also confirmed by T-test (p <0.0001).

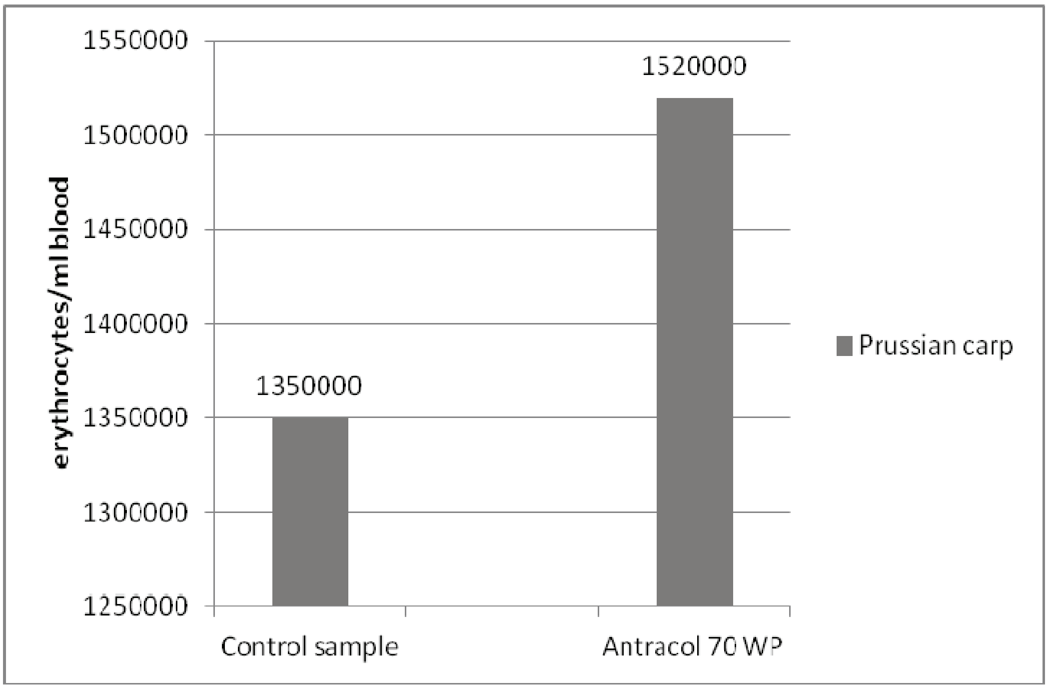

Figure 6. Action of the fungicide Antracol 70 WP at a concentration of 2 mg/l upon the number of erythrocytes at the Prussian carp at 20-22°C.

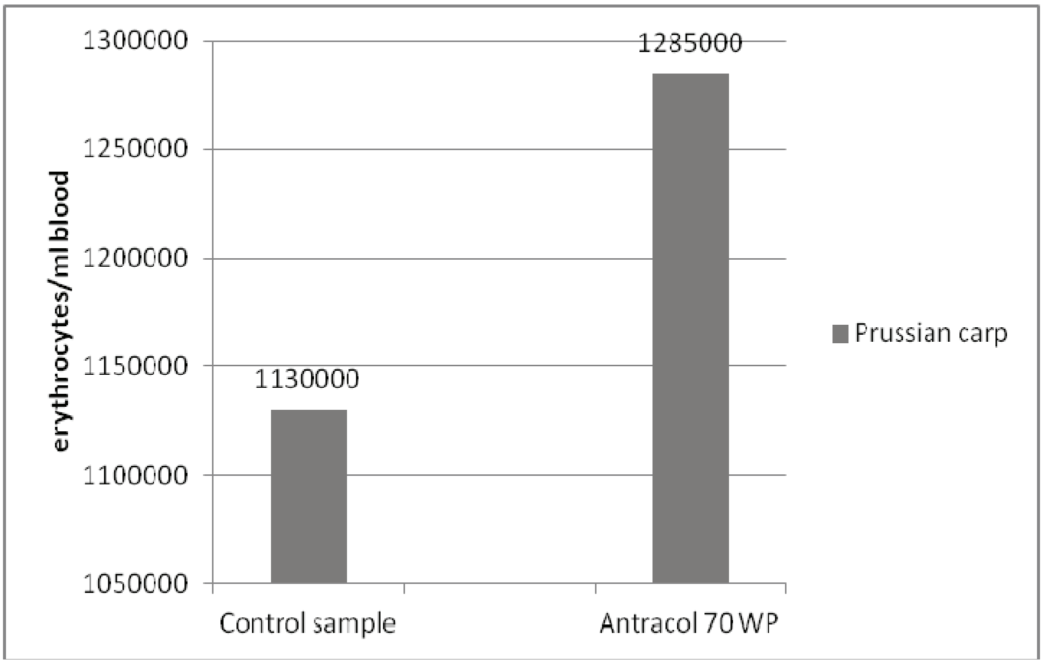

Figure 7. Action of the fungicide Antracol 70 WP at a concentration of 2 mg/l upon the number of erythrocytes at the Prussian carp at 5-7°C.

The percentage increases (compared to control samples) determined after 14 days of exposure to Antracol 70 WP are as follows: 12.6% at a temperature of 20-22°C and 13.7% at a temperature of 5-7°C.

4. The effect of the fungicide Antracol 70 WP upon glycaemia

The application of the fungicide Antracol 70 WP leads to statistically significant results compared to the control groups in terms of blood glucose levels. This is also confirmed by T-test ($p < 0.0001$).

The fungicide Antracol 70 WP at a concentration of 2 mg / l determines the reduction of blood glucose levels by 15% in case of the Prussian carp after two weeks of exposure at 20-22°C (Fig. 8).

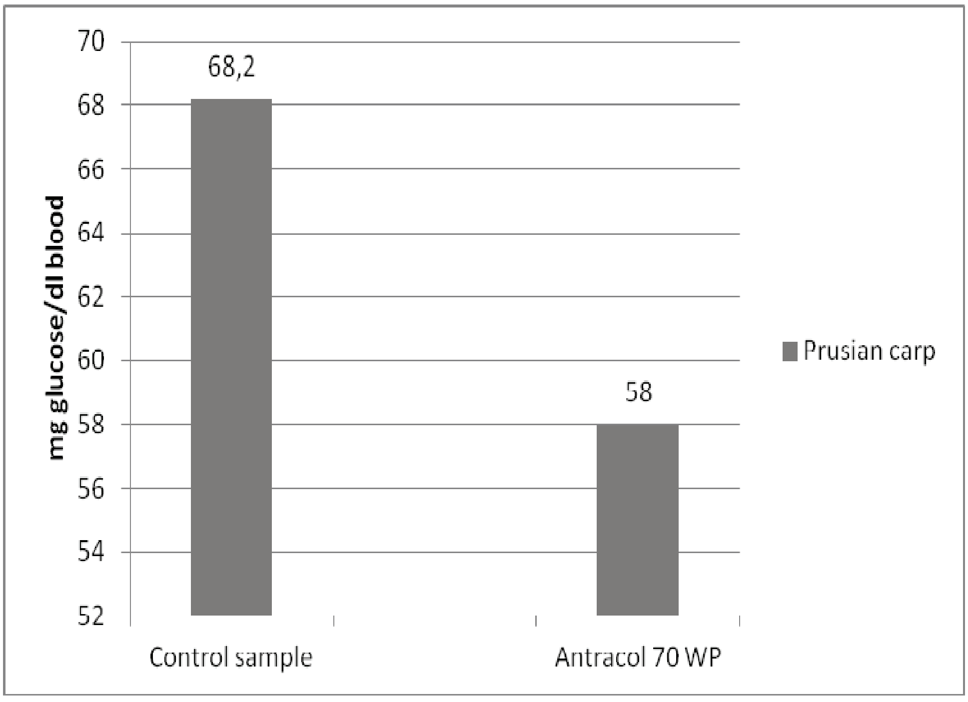

Figure 8. Action of the fungicide Antracol 70 WP at a concentration of 2 mg/l upon blood glucose level at the Prussian carp at 20-22°C.

Low temperature determines the reduction of the glycaemia values by 9.6% at the Prussian carp (Fig. 9).

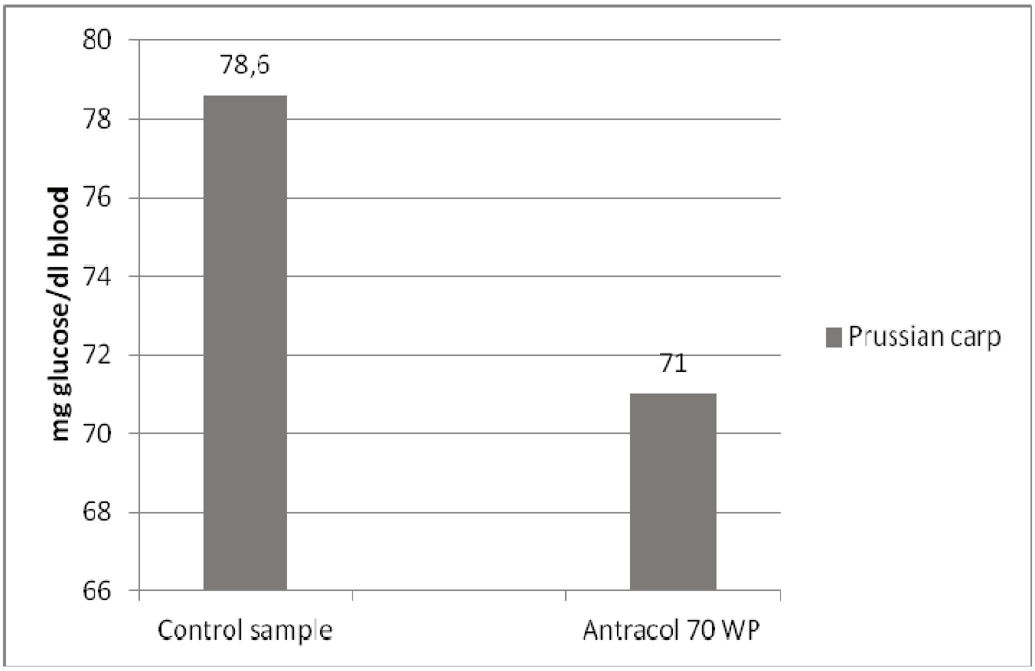

Figure 9. Action of the fungicide Antracol 70 WP at a concentration of 2 mg/l upon blood glucose level at the Prussian carp at 5-7°C.

CONCLUSIONS

The fungicide Antracol 70 WP inhibits the oxygen consumption (28-38% at the temperature of 20-22°C and 30-34% at the temperature of 5-7°C and respiratory rate in the Prussian carp (*Carassius gibelio* Bloch). The number of erythrocytes increases in case of the exposure of fish to the fungicide Antracol 70 WP and blood glucose levels decrease, this fungicide having hypoglycaemic effect. At low temperatures (5-7°C), the toxic effect of the fungicide is slightly weaker compared to that registered at the temperature of 20-22°C.

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